

CLAIMS

1. A chelated complex comprised of (a) a bacteriocin selected from the group consisting of lantibiotics, non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof, and (b) a detectable label comprising a transition or lanthanide metal.
2. The complex of claim 1, wherein the complex binds to microbial cells selected from the group consisting of gram positive bacteria or mycobacteria.
3. The complex of claim 1, wherein the complex binds to gram negative bacteria or fungi.
4. The complex of claim 1, wherein the transition metal is selected from the group consisting of Cu, Co, Fe, Mn, Cr, Ni, Zn, Tc, and their isotopes.
5. The complex of claim 1, wherein the lanthanide metal is selected from the group consisting of Gd, La, Eu, Tb, Dy, and Er.
6. The complex of claim 1, wherein the lantibiotic is selected from the group consisting of nisin, mutacin, subtilin, gallidermin, Pep5, epicidin 280, epilancin K7, lactocin S, streptococcin A-FF22, lacticin 481, salivaricin A, variacin, cypemycin, mersacidin, cinnamycin, duramycin and ancovenin, actagardine, sublancin, plantaricin C, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof.
7. The complex of claim 1, wherein the transition metal is Co.
8. The complex of claim 1, wherein the bacteriocin is selected from the group consisting of nisin, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof.

9. The complex of claim 8, wherein the transition metal is Co or Cr.

10. A method for synthesizing a bacteriocin-metal complex, comprising: (a) admixing
5 (i) a water soluble salt of metal selected from the group consisting of transition metals
and lanthanides with (ii) a bacteriocin selected from the group consisting of lantibiotics,
non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins,
fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof,
in (iii) a solvent for the metal salt and the antibiotic, wherein the admixing is conducted
10 under conditions effective to promote chelation of the metal by the bacteriocin, thereby
forming a solution of the complex of the bacteriocin and the metal;
(b) desalting the complex; and
(c) isolating and drying the complex.

15 11. The method of claim 10, wherein the complex binds to gram positive bacteria or
mycobacteria.

12. The method of claim 10, wherein the complex binds to gram negative bacteria or
fungi.

20 13. The method of claim 10, wherein the solvent comprises aqueous buffer.

14. The method of claim 10, wherein step (b) comprises dialysis.

25 15. The method of claim 10, wherein step (b) comprises gel filtration.

16. The method of claim 10, wherein step (c) comprises freeze-drying.

17. The method of claim 10, wherein step (c) comprises spray drying.

18. A method for forming a bacteriocin-metal complex *in situ* on a sample to be tested, comprising applying to a sample to be tested (i) a water-soluble salt of metal selected from the group consisting of transition metals and lanthanides and (ii) a bacteriocin selected from the group consisting of lantibiotics, non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof, in (iii) a solvent for the metal salt and the bacteriocin.

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10 19. The method of claim 18, wherein the bacteriocin-metal complex binds to a target pathogen.

20. The method of claim 18, wherein the transition metal is selected from the group consisting of Cu, Co, Fe, Mn, Cr, Ni, Zn, Tc, and their isotopes.

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21. The method of claim 18, wherein the lanthanide metal is selected from the group consisting of Gd, La, Eu, Tb, Dy, Er, and their isotopes.

22. The method of claim 18, further comprising contacting the sample with an oxidizable substrate and a source of peroxide and measuring luminescence from the sample.

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23. The method of claim 22, wherein unbound bacteriocin and metal is removed from the sample.

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24. The method of claim 18, wherein a portion of the sample is removed for detection of pathogens.

25. The method of claim 24, wherein the portion of sample is removed by washing.

26. The method of claim 24, wherein the portion of sample is removed and pathogens are suspended in aqueous buffer solution.

27. The method of claim 24, wherein the portion of sample removed for detection of pathogens is concentrated.

28. The method of claim 27, wherein the pathogens are concentrated by a method selected from the group consisting of centrifugation, filtration or adsorption.

10 29. The method of claim 28, wherein the adsorption is performed by adsorptive particles selected from the group consisting of immuno-microbeads and phage-microbeads.

15 30. The method of claim 22, wherein the oxidizable substrate is selected from the group of chemiluminescent substrates consisting of luminol and its derivatives, lucigenin, penicillin, luciferin and other polyaromatic phthalylhydrazides.

31. The method of claim 22, wherein the peroxide source is hydrogen peroxide, benzoyl peroxide or cumyl peroxide.

20 32. The method of claim 22, wherein the peroxide source is an enzyme such as glucose oxidase or amino acid oxidase.

33. A diagnostic test for conducting a chemiluminescent assay of bacteria or fungi, comprising: the complex of claim 1, a peroxide source and oxidizable substrate.

25 34. The diagnostic test of claim 33, wherein the oxidizable substrate is selected from the group of chemiluminescent substrates consisting of luminol and its derivatives, lucigenin, penicillin, luciferin and other polyaromatic phthalylhydrazides.

35. The diagnostic test of claim 33, wherein the peroxide source is hydrogen peroxide, benzoyl peroxide or cumyl peroxide.

36. The diagnostic test of claim 33, wherein the peroxide source is an enzyme such as 5 glucose or amino acid oxidase.

37. The diagnostic test of claim 33, wherein the bacteria are gram positive bacteria, gram negative bacteria or mycobacteria.

10 38. The diagnostic test of claim 33, wherein fungi are detected.

15 39. A method for conducting a chemiluminescent assay of pathogens comprising (a) contacting a sample with the complex of claim 1, (b) removing unbound complex and (c) detecting pathogens by contacting the sample with a peroxide source and an oxidizable substrate.

40. The method of claim 39, wherein pathogens are isolated from the sample prior to contacting the sample with the chelated complex .

20 41. The method of claim 39, wherein pathogens are isolated from the sample using antibody-attached microbeads or phage-attached microbeads.

42. The method of claim 39, wherein the microbeads comprise a magnetic material.

25 43. The diagnostic test of claim 33, further comprising combining bacteria or fungi labeled with the chelated complex of claim 1 with peroxide with an oxidizable substrate, and detecting light emission in a photodetector.

100-200-300-400-500-600-700-800-900-1000

44. The method of claim 39, wherein the peroxide source is hydrogen peroxide, benzoyl peroxide and cumyl peroxide.

45. The method of claim 39, wherein the oxidizable substrate is selected from the group 5 consisting of luminol and its derivatives, lucigenin, penicillin, luciferin and other polyaromatic phthalylhydrazides.

46. The method of claim 39, wherein the pathogens are gram positive bacteria or mycobacteria.

10 47. The method of claim 39, wherein the pathogens are gram negative bacteria or fungi.

15 48. A therapeutic treatment comprising a chelated complex comprised of (a) lantibiotics, non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof, and (b) a detectable label comprising a transition or lanthanide metal, wherein the tissue of a patient is treated with the chelated complex.

20 49. The therapeutic treatment of claim 48, wherein the transition metal is Cobalt.

50. The therapeutic treatment of claim 48, wherein the lantibiotic is nisin.

25 51. The diagnostic test of claim 33, wherein the bacteria are selected from the group consisting of lactococci, leuconostocs, micrococci, pediococci, actinomyces, mycobacteria, pneumococci, streptococci, staphylococci, aerobic bacilli, anaerobic clostridia, listeria and nocardia.

52. The diagnostic test of claim 51, wherein the mycobacteria are selected from the group consisting of *mycobacterium tuberculosis*, *mycobacterium avium*, *mycobacterium paratuberculosis*, *mycobacterium bovis* and *mycobacterium leprae*.

5 53. The diagnostic test of claim 51, wherein the bacteria are selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum* and *Clostridium perfringes*.

10 54. The method of claim 39, wherein the bacteria are selected from the group consisting of lactococci, leuconostocs, micrococci, pediococci, actinomyces, mycoabacteria, pneumococci, streptococci, staphylococci, aerobic bacilli, anaerobic clostridia, listeria and nocardia.

15 55. The method of claim 54, wherein the mycobacteria are selected from the group consisting of *mycobacterium tuberculosis*, *mycobacterium avium*, *mycobacterium paratuberculosis*, *mycobacterium bovis* and *mycobacterium leprae*.

56. The method of claim 54, wherein the bacteria are selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum* and *Clostridium perfringes*.

20 57. A method for synthesizing a lantibiotic -metal complex, comprising
(a) admixing (i) a water soluble salt of metal selected from the group consisting of transition metals and lanthanides with (ii) a lantibiotic selected from the group consisting of nisin, mutacin, subtilin, gallidermin, Pep5, epicidin 280, epilancin K7, lactocin S, streptococcin A-FF22, lacticin 481, salivaricin A, variacin, cypemycin, mersacidin, cinnamycin, duramycin and ancovenin, actagardine, sublancin, plantaricin C, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof, in (iii) a solvent for the metal salt and the lantibiotic, wherein the admixing is conducted under conditions

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effective to promote chelation of the metal by the lantibiotic, thereby forming a solution of the complex of the lantibiotic and the metal;

- (b) desalting the complex; and
- (c) isolating and drying the complex.

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58. The method of claim 57, wherein the solvent comprises aqueous buffer.

59. The method of claim 57, wherein step (b) comprises dialysis.

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60. The method of claim 57, wherein step (b) comprises gel filtration.

61. The method of claim 57, wherein step (c) comprises freeze drying.

62. The method of claim 57, wherein step (c) comprises spray drying.

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63. The complex of claim 1, wherein the lantibiotic is selected from the group consisting of nisin, mutacin, subtilin, gallidermin, Pep5, epicidin 280, epilancin K7, lactocin S, streptococcin A-FF22, lacticin 481, salivaricin A, variacin, cypemycin, mersacidin, cinnamycin, duramycin and ancovenin, actagardine, sublancin, plantaricin C, mixtures thereof and fragments, analogs and variants thereof, and the lanthanide metal is selected from the group consisting of Gd, La, Eu, Tb, Dy, and Er, and their isotopes.

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64. The complex of claim 1, wherein the lantibiotic is selected from the group consisting of nisin, mutacin, subtilin, gallidermin, Pep5, epicidin 280, epilancin K7, lactocin S, streptococcin A-FF22, lacticin 481, salivaricin A, variacin, cypemycin, mersacidin, cinnamycin, duramycin and ancovenin, actagardine, sublancin, plantaricin C, mixtures thereof and fragments, analogs and variants thereof, and the transition metal is selected from the group consisting of Cu, Co, Fe, Mn, Cr, Ni, Zn, Tc, and their isotopes.

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65. The complex of claim 1, wherein the bacteriocin comprises the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:8 or a nucleic acid sequence that hybridizes with SEQ ID NO:8 under stringent conditions.

5 66. The complex of claim 1, wherein the bacteriocin comprises the amino acid sequence of SEQ ID NOS: 1-7, or the amino acid sequence of SEQ ID NOS: 1-7 having a substitution, deletion or addition of 1 to 3 amino acids.

10 67. The complex of claim 1, wherein the bacteriocin comprises the amino acid sequence of SEQ ID NOS: 1-7 or an amino acid sequence that is 90% homologous with the amino acid sequence of SEQ ID NOS: 1-7.

68. A method for forming a bacteriocin-metal complex *in situ* on a sample to be tested, comprising applying to a sample to be tested:

15 (i) a water-soluble salt of metal selected from the group consisting of transition metals and lanthanides and

(ii) a bacteriocin, wherein the bacteriocin comprises the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:8 or a nucleic acid sequence that hybridizes with SEQ ID NO:8 under stringent conditions; in

20 (iii) a solvent for the metal salt and the bacteriocin.

69. A method for forming a bacteriocin-metal complex *in situ* on a sample to be tested, comprising applying to a sample to be tested:

25 (i) a water-soluble salt of metal selected from the group consisting of transition metals and lanthanides and

(ii) a bacteriocin, wherein the bacteriocin comprises the amino acid sequence of SEQ ID NOS: 1-7, or the amino acid sequence of SEQ ID NOS: 1-7 having a substitution, deletion or addition of 1 to 3 amino acids; in

(iii) a solvent for the metal salt and the bacteriocin.

70. A method for forming a bacteriocin-metal complex *in situ* on a sample to be tested, comprising applying to a sample to be tested:

- 5 (i) a water-soluble salt of metal selected from the group consisting of transition metals and lanthanides and
- (ii) a bacteriocin, wherein the bacteriocin comprises the amino acid sequence of SEQ ID NOS: 1-7 or an amino acid sequence that is 90% homologous with the amino acid sequence of SEQ ID NOS: 1-7; in
- (iii) a solvent for the metal salt and the bacteriocin.

10 71. A method for conducting a chemiluminescent agglutination assay for an analyte comprising

- (a) providing *Staphylococcus aureus* cells with antibodies to the analyte bound thereto,
- 15 (b) contacting a sample with the *Staphylococcus* cells,
- (c) allowing the antibodies to bind to the analyte and agglutinate the *Staphylococcus* cells,
- (d) separating the agglutinated cells from the non-agglutinated cells,
- (e) contacting the agglutinated cells with a bacteriocin and a transition or

20 lanthanide metal,

- (f) optionally removing unbound complex and
- (g) detecting the presence of the analyte by contacting the sample with a peroxide source and an oxidizable substrate.

25 72. A method for conducting a chemiluminescent agglutination assay for viruses or prions comprising

- (a) providing *Staphylococcus aureus* cells with antibodies to viruses or prions bound thereto,
- (b) contacting a sample with the *Staphylococcus* cells,

(c) allowing the antibodies to bind to viruses or prions and agglutinate the *Staphylococcus* cells,

(d) separating the agglutinated cells from non-agglutinated cells,

(e) contacting the agglutinated cells with a bacteriocin and a transition or lanthanide metal,

(f) optionally removing unbound complex and

(g) detecting the presence of viruses or prions by contacting the sample with a peroxide source and an oxidizable substrate.

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